REMARKS

I. Status of the Claims

Claims 44-57 are pending in the application and stand rejected under the first and second paragraphs of 35 U.S.C. §112. The specific grounds for rejection, and applicants' response thereto, are set out in detail below.

II. Sequence Compliance

Applicants note the examiner's comments on unfulfilled sequence requirements for FIG. 1A and page 65. Applicants have amended the specification to meet sequence requirements and are enclosing same on disk.

III. Rejection Under 35 U.S.C. §112, Second Paragraph

Claim 44 (and claims depending therefrom) is rejected for use of the term "said protein" in the last line as that phrase does not identify to which protein the phrase refers. A clarifying amendment is provided. Reconsideration and withdrawal of the rejection is therefore respectfully requested.

IV. Rejection Under 35 U.S.C. §112, First Paragraph

Claims 44-57 are rejected under §112, first paragraph, as allegedly lacking an enabling disclosure. The examiner's concerns are (a) that the claims are not enabled for assessing protein stability, and (b) that the claims are not enabled for *in vitro* assessment using purified proteins. Applicants traverse.

A. Protein Stability

According to the examiner, the specification contains insufficient teachings to instruct one of ordinary skill in the art how to assess protein stability in the context of the present invention. The crux of the examiner's concerns derive from the following:

> .... To determine the stability of a protein, one must measure a known amount of a protein with regard to its conformation or assembly over time to monitor for any decrease in assembly over than time period. This type of experiment would normally be performed utilizing some sort of pulse-chase experiment such that a particular subset of proteins could be analyzed over time. The nature of this assay is not accounted for in the present invention because the examples given in the specification all measure the structural complementation that occurs at [a] fixed point in time, essentially taking a snapshot of the structural considerations of the protein of interest.

Thus, it seems as if the primary issue is whether the specification supports time-course examination of protein stability, and the assay formats to support it.

Applicants submit that stability, just like folding and solubility, can be assessed using precisely the same assay as described. It is important to recall that prior to lysis and assay, the target fusion protein is expressed in a living cell, and is therefore subject to surveillance and evaluation by the protein quality control machinery functioning in that cellular environment during expression. In consequence, thermodynamic or biochemical instability of the target protein owing to mutation, different expression conditions, or other environmental insult would be expected to result in sequestration, insoluble aggregation, or accelerated turnover by proteolysis (thermodynamic stability being defined by the relative populations of folded and unfolded protein at equilibrium). Biochemical stability is defined as the relative rate of turnover as compared to production, which can be held constant. In each case, the result is a decrease in the steady-state level of the target fusion protein and a corresponding reduction in marker protein activity. It is not necessary – as argued by the examiner – that one assess stability over any time

period. Rather, one simply can examine the amount of marker protein activity that is produced in the assay at fixed time point, and correlate this with some protein abnormality, be it one of folding, stability or solubility. Admittedly, the assay does not permit one to distinguish ***between*** solubility, folding or stability, but rather, only that the protein produced by a cell has one or more of these defects. Regardless, each will result in a reduction in complementation, and therefore a reduction in signal. That is all that is required for the assay to be performed and interpreted. Furthermore, by treating the cell to inhibit protein synthesis and then taking multiple readings from treated cells over time, the assay can be used to measure the rate of loss of soluble target protein. Indeed, the assay has been successfully used to monitor changes in the level of soluble beta-amyloid over time, as discussed in Wigley *et al.* (*Nature Biotechnology*, 2001 Feb;19(2):131-136; see p. 134).

In light of these statement, applicants respectfully submit that, just like folding and solubility, protein stability can be assessed using the present assay.

B. Cell Free Systems

The examiner also argues that the specification, though replete with a description of cell based assays using recombinant expression of fusion proteins, fails to teach how to make and use a cell-free system, and to deal with problems attendant therewith. For example, the examiner suggests that many of the fusion proteins will be insoluble, and hence unusable in cell free formats. Applicants traverse

The examiner seems to miss the point of applicants' assay in arguing that insolubility will prevent the assay from functioning as intended. Indeed, the presence of an insoluble recombinant protein, as discussed in the specification, will prevent complementation and

therefore result in reduced or no signal. ***But this is the point of the assay***. The lack of soluble protein that is available for complementation– due to instability, improper folding or some other reason – will indicate a defect in the recombinant protein produced by the cell. Thus, a reduced signal is precisely what is expected when protein is found to be insoluble, and this will occur in either a cell-based or cell-free system. So long as techniques well known in the art are used to provide an appropriate milieu of ionic strength and composition, pH, *etc.*, the status of the target fusion protein after cell lysis will reflect its status prior to cell lysis. Therefore, if significant amounts of properly folded, soluble target fusion protein are present in cells, the lysate will also contain significant amounts of properly folded, soluble target fusion. Moreover, this feature of the assay enables the identification of compounds or conditions that positively influence the level of soluble target fusion protein in the cell because such an effect will result in an increased enzymatic signal after cell lysis. Because the structural complementation reaction is a reversible reaction that reaches equilibrium, there is no inherent difference to be expected between the degree of complementation achievable when both marker fragments are expressed in the same cell versus when only the target fusion is expressed and the complementation partner is added after cell lysis. The lysis of the cells may cause a fractional loss of target fusion protein, but as long as the conditions are consistent this loss will be similar in both cases. The degree of structural complementation in the lysate will accurately reflect the levels of properly folded, soluble target protein so long as an adequate amount of the complementation partner is provided. Again, this may be achieved by methods that are well known and readily apparent to the skilled practitioner. The expression of both the target fusion and the complementation partner in the same cell, as described in the specification, is a matter of convenience and efficiency rather than a requirement for technical success.

In short, the very premise of the examiner's rejection assumes an ***operable*** assay – one where the insolubility of a protein takes it away from the pool of molecules capable of α-complementation. If a loss of soluble protein is observed, due to any cause (misfolding, insolubility, increased turnover, sequestration, *etc.*) then applicants' assay will show this. Therefore, it is respectfully submitted that the claimed invention is indeed operable in cell-free formats.

V. Obviousness-Type Double-Patenting

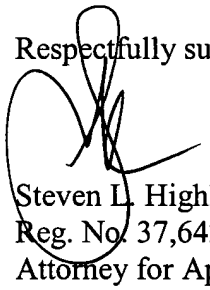
Claims 44-57 are rejected as obvious over claims 1-13 of U.S. Patent 6,727,070. Applicants are filing herewith a terminal disclaimer to obviate the rejection without in any way acquiescing to the rejection. Reconsideration and withdrawal of the rejection is therefore respectfully requested.

IV. Conclusion

In light of the foregoing, applicants respectfully submit that all claims are in condition for allowance, and an early notification to that effect is earnestly solicited. Any questions regarding this response may be directed to the undersigned at the telephone number provided below.

Please date stamp and return the enclosed postcard as evidence of receipt.

Respectfully submitted,



Steven L. Highlander
Reg. No. 37,642
Attorney for Applicants

FULBRIGHT & JAWORSKI L.L.P.
600 Congress Avenue, Suite 2400
Austin, Texas 78701
(512) 536-3184

Date: May 23, 2005